

## Supplemental Table I

### Plasmids used in this study

Plasmids	Description	Source
<b>Gateway cloning vectors</b>		
pENTR1A, -2B and -3C	Gateway entry vectors	Invitrogen
pDest15	Bacterial GST fusion expression vector; T7 promoter	Invitrogen
pDEST-TH1	Bacterial MBP fusion expression vector; tac promoter	(1)
pDestEGFP-C1	Mammalian EGFP fusion expression vector; CMV promoter	(2)
pDest-mCherry-C1	Mammalian mCherry fusion expression vector, backbone as pDestEGFP-C1	(3)
pDest-3xFLAG	Mammalian triple FLAG-tag fusion expression vector; CMV promoter	(4)
pDest-myc	Mammalian myc-tag fusion expression vector; backbone as pcDNA3.1	(2)
pDest53	Mammalian GFP fusion expression vector, CMV and T7 promoter	Invitrogen
pDestEGFP-Flp-In-FRT/TO	EGFP gene and downstream gateway cassette subcloned from pDestEGFP-C1 into polylinker of pcDNA5/FRT/TO	This study
<b>Other vectors</b>		
pGEX-2T	Bacterial GST fusion expression vector; tac promoter	Amersham
pcDNA5/FRT/TO	Mammalian Flp-In expression vector; Tet-inducible CMV promoter	Invitrogen
<b>cDNA constructs made by traditional subcloning or site-directed mutagenesis</b>		
pENTR223.1-ULK1	Human ULK1 in Gateway entry vector (HsCD00082595)	DF/HCC DNA Resource Core, Harvard
pENTR-ULK1 F357A	Made by site-directed mutagenesis of pENTR223.1-ULK1	This study
pENTR-ULK1 F357A/V360A	Made by site-directed mutagenesis of pENTR223.1-ULK1	This study
pENTR-ULK1(1-350)	ULK1(1-350) in Gateway entry vector	This study
pENTR-ULK1(1-370)	ULK1(1-370) in Gateway entry vector	This study
pENTR-ULK1(371-1050)	ULK1(371-1050) in Gateway entry vector	This study
pENTR-ULK1(351-400)	ULK1(351-400) in Gateway entry vector	This study
pENTR-ULK1(351-370)	ULK1(351-370) in Gateway entry vector	This study
pENTR-ULK1(351-400) point mutants (F357A, F357A/V360A)	Made by site-directed mutagenesis of pENTR-ULK1(351-400)	This study
pcDNA3.1-ATG13-FLAG	Human ATG13, C-terminally tagged with FLAG, in pcDNA3.1	(5)
pENTR-ATG13	ATG13 from pcDNA3.1-ATG13-FLAG in Gateway entry vector	This study
pENTR-ATG13 F444A	Made by site-directed mutagenesis of pENTR-ATG13	This study
pENTR-ATG13 F444A/I447A	Made by site-directed mutagenesis of pENTR-ATG13	This study
pENTR-ATG13(1-457)	ATG13(1-457) in Gateway entry vector	This study
pENTR-ATG13(1-437)	ATG13(1-437) in Gateway entry vector	This study
pENTR-ATG13(438-517)	ATG13(438-517) in Gateway entry vector	This study
pENTR-ATG13(438-457)	ATG13(438-457) in Gateway entry vector	This study
pENTR-ATG13(458-517)	ATG13(458-517) in Gateway entry vector	This study
pDONR221-FIP200	Human FIP200 in Gateway DONR vector (HsCD00044465)	DF/HCC DNA Resource Core, Harvard
pENTR-LC3A	Human LC3A in Gateway entry vector	(3)
pENTR-GABARAP	Human GABARAP in Gateway entry vector	(3)
pENTR-GABARAP point mutants (K46A, K48A, Y49A, L50A, R67A, F77A)	Made by site-directed mutagenesis of pENTR-GABARAP	This study
pENTR-GABARAP(1-26)	Human GABARAP(1-26) in Gateway entry vector	This study
pENTR-GABARAP(28-117)	Human GABARAP(28-117) in Gateway entry vector	This study
pENTR-GABARAPL1	Human0 GABARAPL1 in Gateway entry vector	(3)
pDONR221-yAtg1	Yeast Atg1 in gateway donor vector (HsCD00025090)	DF/HCC DNA Resource Core, Harvard
pDONR201-yAtg8	Yeast Atg8 in gateway donor vector (HsCD00011665)	DF/HCC DNA Resource Core, Harvard
pUASp-GFP-Atg8A	Drosophila Atg8A in pUASp-hrGFP	(6)
pENTR-DmAtg8A	Drosophila Atg8A from pUASp-GFP-Atg8A in Gateway entry vector	This study
pDONR221-DmAtg1B	Drosophila Atg1B was isolated by PCR using cDNA made from RNA isolated from S2R+ cells, and then inserted into pDONR221 by Gateway BP reaction	This study
pDONR221-DmAtg1B point mutants (F391A, V394A, F391A/V394A)	Made by site-directed mutagenesis of pDONR221-Atg1B	This study
pENTR-FYCO1(1276-1294)	Human FYCO1 in Gateway entry vector	This study
pGEX4T1-LC3C(ΔG)	Human LC3C in pGEX4T1	(7)
pEGFP-C1-LC3C(ΔG)	Human LC3C in pEGFP-C1	(7)
<b>cDNA constructs made by Gateway LR reactions</b>		
pDest15-ULK1(351-400)	ULK1(351-400) in pDest15	This study
pDest15-ULK1(351-400) F357A	ULK1(351-400) F357A in pDest15	This study
pDest15-ULK1(351-400) F357A/V360A	ULK1(351-400) F357A/V360A in pDest15	This study

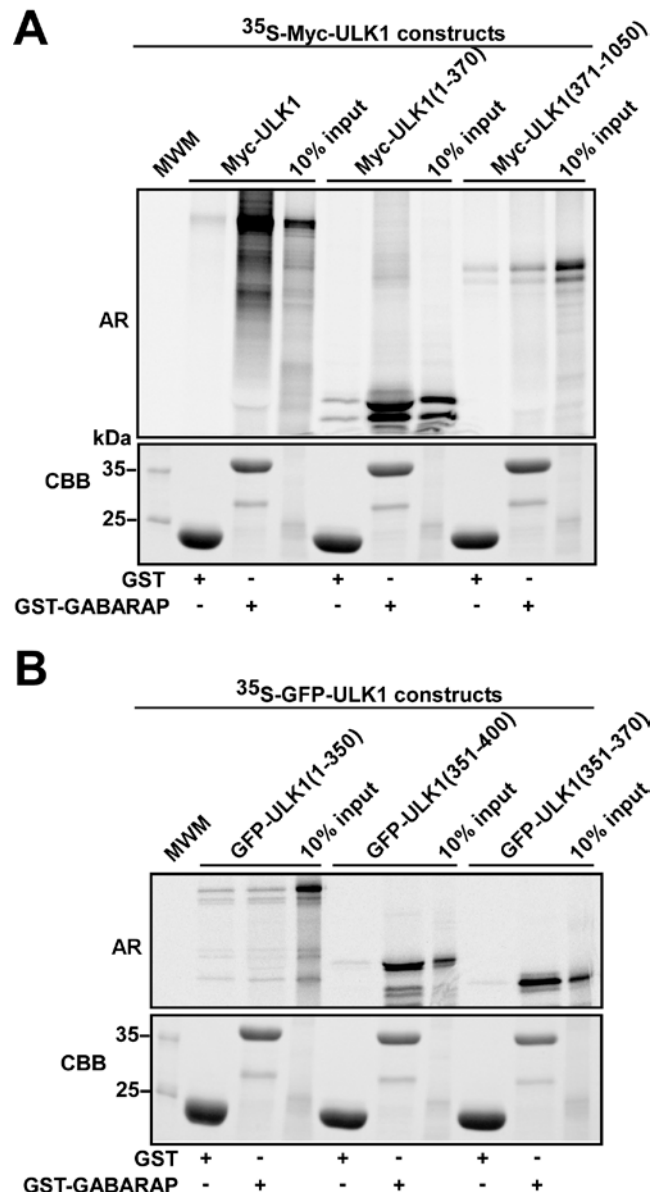
pDest15-ATG13	ATG13 in pDest15	This study
pDest15-LC3A	LC3A in pDest15	(3)
pDest15-LC3B	LC3B in pDest15	(3)
pDest15-GABARAP	GABARAP in pDest15	(3)
pDest15-GABARAPL1	GABARAPL1 in pDest15	(3)
pDest15-GABARAPL2	GABARAPL2 in pDest15	(3)
pDest15-GABARAP(1-26)	GABARAP(1-26) in pDest15	This study
pDest15-GABARAP(28-117)	GABARAP(28-117) in pDest15	This study
pDest15-yAtg8	Yeast Atg8 in pDest15	This study
pDest15-DmAtg8A	Drosophila Atg8A in pDest15	This study
pDest15-GABARAP K46A	GABARAP K46A in pDest15	This study
pDest15-GABARAP K48A	GABARAP K48A in pDest15	This study
pDest15-GABARAP Y49A	GABARAP Y49A in pDest15	This study
pDest15-GABARAP L50A	GABARAP L50A in pDest15	This study
pDest15-GABARAP R67A	GABARAP R67A in pDest15	This study
pDest15-GABARAP F77A	GABARAP F77A in pDest15	This study
pDest-TH1-GABARAP	GABARAP in pDest-TH1	This study
pDestEGFP-LC3A	LC3A in pDestEGFP-C1	This study
pDestEGFP-LC3B	LC3B in pDestEGFP-C1	(7)
pDestEGFP-GABARAP	GABARAP in pDestEGFP-C1	This study
pDestEGFP-GABARAPL1	GABARAPL1 in pDestEGFP-C1	This study
pDestEGFP-GABARAPL2	GABARAPL2 in pDestEGFP-C1	(7)
pDest-3xFLAG-ULK1	ULK1 in pDest-3xFLAG	This study
pDest-3xFLAG-ULK1 F357A/V360A	ULK1 F357A/V360A in pDest-3xFLAG	This study
pDest-3xFLAG-GABARAP	GABARAP in pDest-3xFLAG	This study
pDest-mCherry-ULK1	ULK1 in pDest-mCherry	This study
pDest-mCherry-ULK1 F357A/V360A	ULK1 F357A/V360A in pDest-mCherry	This study
pDest-myc-ULK1	ULK1 in pDest-myc	This study
pDest-myc-ULK1(1-370)	ULK1(1-370) in pDest-myc	This study
pDest-myc-ULK1(371-1050)	ULK1(371-1050) in pDest-myc	This study
pDest-myc-ULK1 F357A	ULK1 F357A in pDest-myc	This study
pDest-myc-ULK1 F357A/V360A	ULK1 F357A/V360A in pDest-myc	This study
pDest-myc-ATG13	ATG13 in pDest-myc	This study
pDest-myc-FIP200	FIP200 in pDest-myc	This study
pDest-myc-yAtg1	Yeast Atg1 in pDest-myc	This study
pDest-myc-DmAtg1B	Drosophila Atg1B in pDest-myc	This study
pDest-myc-DmAtg1B F391A	Drosophila Atg1B F391A in pDest-myc	This study
pDest-myc-DmAtg1B V394A	Drosophila Atg1B V394A in pDest-myc	This study
pDest-myc-DmAtg1B F391A/V394A	Drosophila Atg1B F391A/V394A in pDest-myc	This study
pDest53-ULK1(1-350)	ULK1(1-350) in pDest53	This study
pDest53-ULK1(351-400)	ULK1(351-400) in pDest53	This study
pDest53-ULK1(351-370)	ULK1(351-370) in pDest53	This study
pDest53-ATG13	ATG13 in pDest53	This study
pDest53-ATG13(1-457)	ATG13(1-457) in pDest53	This study
pDest53-ATG13(1-437)	ATG13(1-437) in pDest53	This study
pDest53-ATG13(438-517)	ATG13(438-517) in pDest53	This study
pDest53-ATG13(438-457)	ATG13(438-457) in pDest53	This study
pDest53-ATG13(458-517)	ATG13(458-517) in pDest53	This study
pDest53-ATG13 F444A	ATG13 F444A in pDest53	This study
pDest53-ATG13 F444A/I447A	ATG13 F444A/I447A in pDest53	This study
pDest53-GABARAP	GABARAP in pDest53	This study
pDest53-FYCO1(1276-1294)	Human FYCO1 LIR motif in pDest53	This study
pDest53-p62(321-349)	Human p62 LIR motif in pDest53	(3)
pDestEGFP-Flp-In-FRT/TO-ULK1	Human ULK1 in pDestEGFP-Flp-In-FRT/TO	This study
pDestEGFP-Flp-In-FRT/TO-ULK1 F357A/V360A	Human ULK1 F357A/V360A in pDestEGFP-Flp-In-FRT/TO	This study

## References

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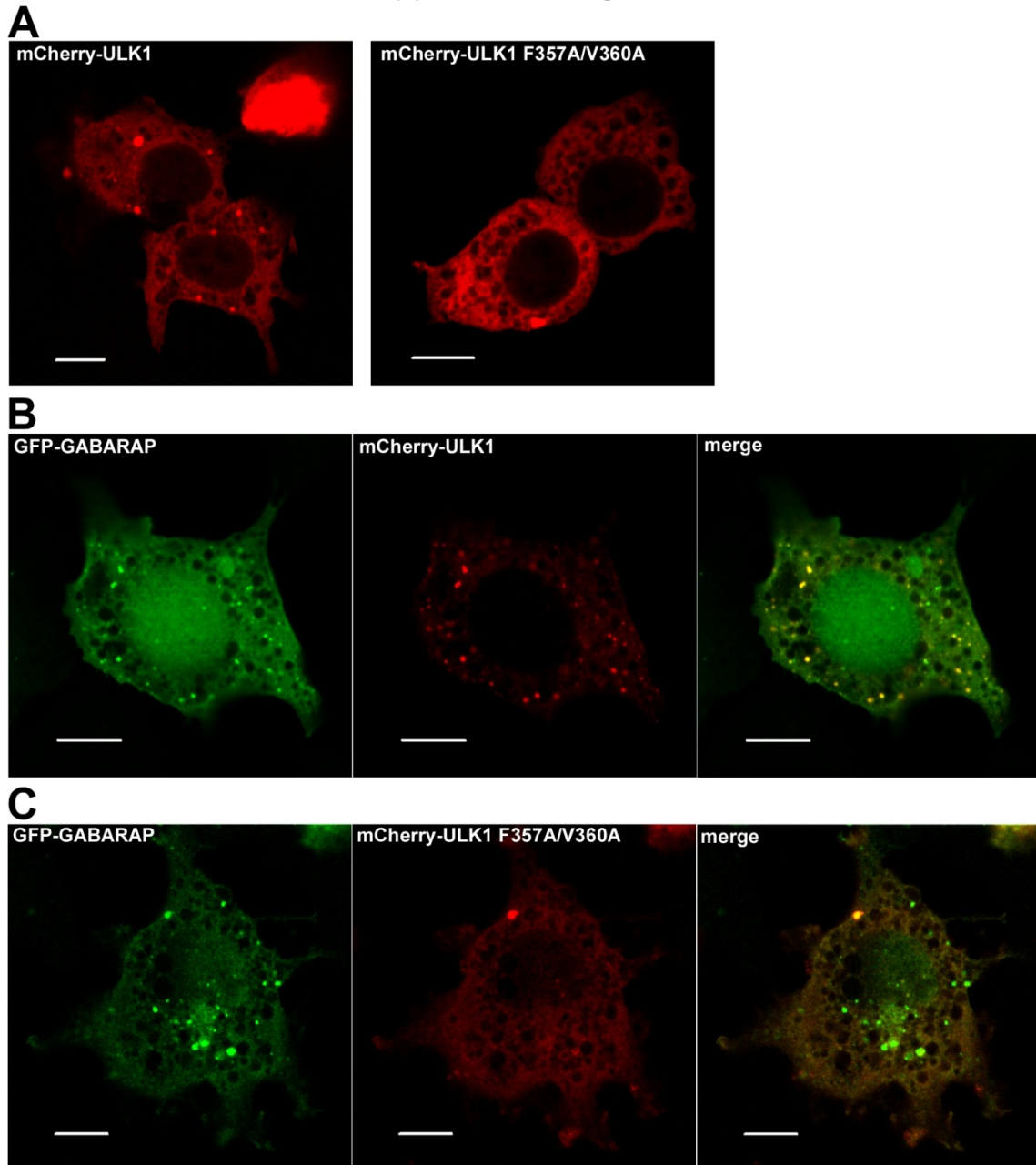
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## Supplemental Figure 1



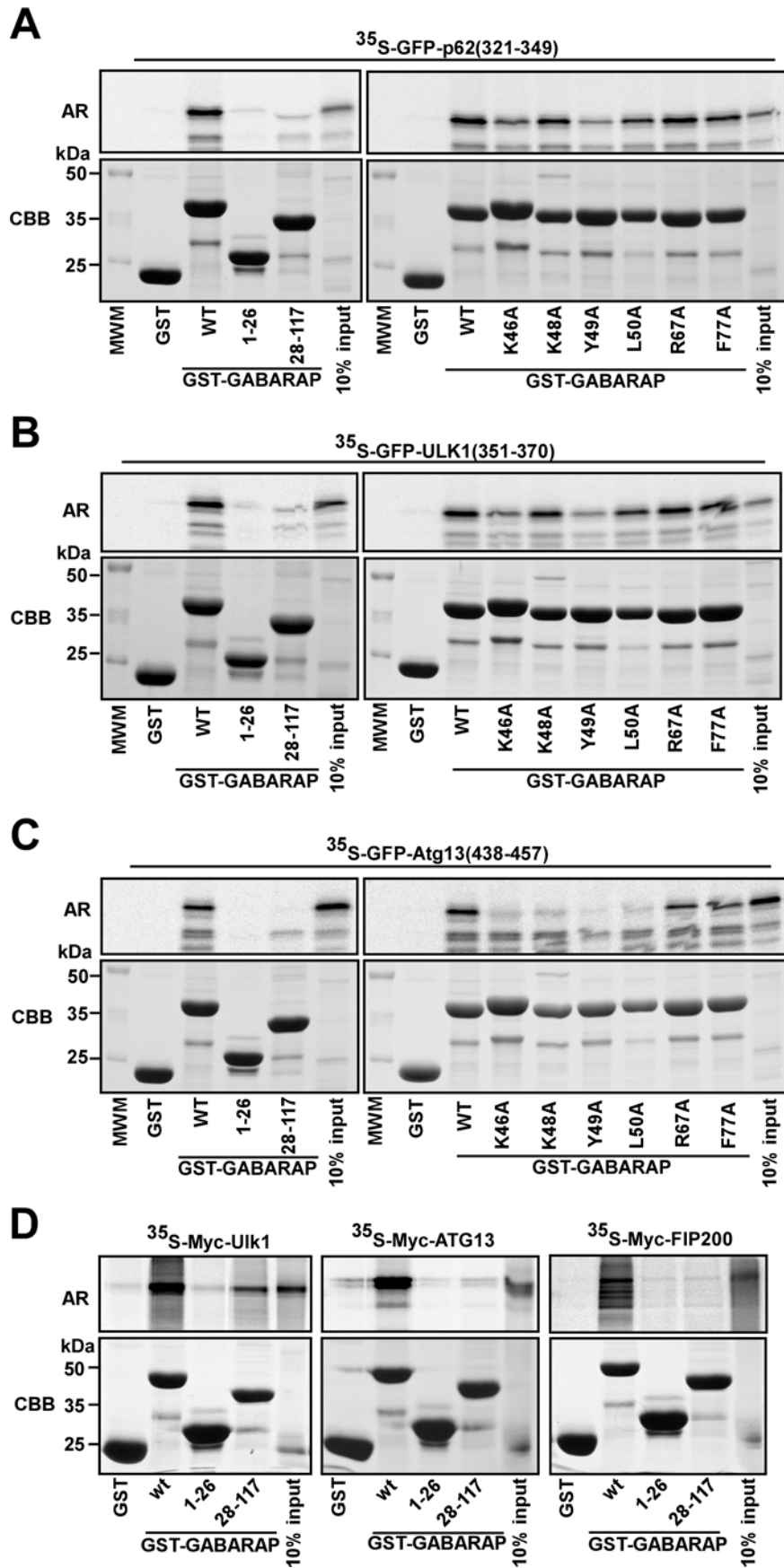
**Supplemental Figure 1.** Amino acids 351-370 of ULK1 are required for GABARAP binding. (A) and (B). The indicated ULK1 constructs were *in vitro* translated in the presence of [<sup>35</sup>S]methionine and tested in GST pull-down assays for binding to recombinant GST or GST-GABARAP. Autoradiographs (AR, upper panels) and Coomassie staining of immobilized GST or GST fusion proteins (bottom panels) are shown.

## Supplemental Figure 2



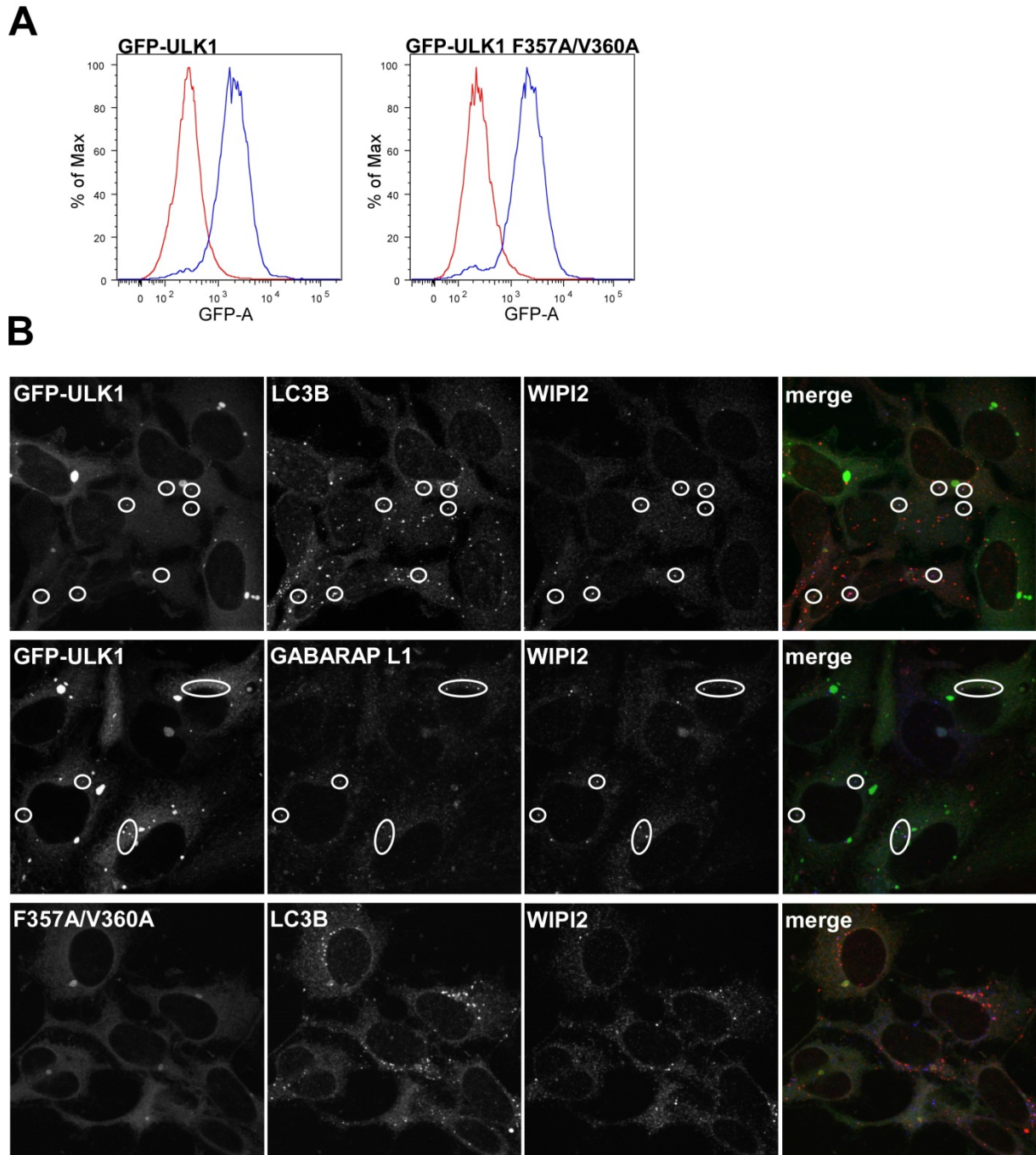
**Supplemental Figure 2.** The LIR motif in ULK1 is needed for efficient co-localisation of overexpressed ULK1 with GABARAP in co-transfected cells. (A-C). HEK293 cells were transfected with mCherry-tagged ULK1 (wild-type or F357/V360 point mutations), either alone (A) or together with GFP-tagged GABARAP (B and C). Cells were analyzed by confocal microscopy 24 h after transfection. Bars, 10  $\mu$ m.

### Supplemental Figure 3



**Supplemental Figure 3.** The interaction of GABARAP with ULK1, ATG13, and FIP200 involve both the C-terminal ubiquitin-like domain and the N-terminal extensions. **(A-C)**. GFP fusions of the indicated LIR motifs from p62, ULK1, and ATG13 were *in vitro* translated and tested for binding to GST or the indicated GST-GABARAP constructs in GST pulldown assays. **(D)**. Full-length ULK1, ATG13, and FIP200 were *in vitro* translated and tested for binding to GST or the indicated GST-GABARAP constructs in GST pulldown assays. **(A-D)**. Autoradiographs (AR, upper panels) and Coomassie staining of immobilized GST or GST fusion proteins (bottom panels) are shown.

## Supplemental Figure 4



**Supplemental Figure 4.** (A). Flow cytometry diagrams showing equal expression levels of wild type and LIR-mutated GFP-ULK1 in HEK293 Flp-In T-Rex cells. Expression of GFP-ULK1 (wild type or mutated) was induced for 24 hours with tetracycline. (B). Triple co-localization of GFP-ULK1, endogenous WIPI2, and endogenous LC3B or GABARAP L1 in dots of HEK293 Flp-In T-Rex cells expressing wild type GFP-ULK1. Expression of GFP-ULK1 (wild type or mutated) was induced for 24 hours with tetracycline. Cells were fixed and stained as indicated with antibodies against LC3B, GABARAP L1, and WIPI2. Cells were then analysed by confocal microscopy. Representative images are shown, the circles indicating dots with triple co-localization.